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(54) Title: IMMUNOSUPPRESSANT COMPOUNDS AND COMPOSITIONS

(57) Abstract: The present invention relates to immunosuppressant, process for their production, their uses and pharmaceutical compositions containing them. The invention provides a novel class of compounds useful in the treatment or prevention of diseases or disorders mediated by lymphocyte interactions, particularly diseases associated with EDG receptor mediated signal transduction.



WO 2004/113330 A1

## IMMUNOSUPPRESSANT COMPOUNDS AND COMPOSITIONS

### CROSS-REFERENCE TO RELATED APPLICATIONS

5           This application claims the benefit of priority to U.S. Provisional Patent Application Number 60/471,931 (filed 19 May 2003) and U.S. Provisional Patent Application Number 60/562,182 (filed 14 April 2004). The full disclosures of these applications are incorporated herein by reference in their entirety and for all purposes.

### BACKGROUND OF THE INVENTION

#### 10    Field of the Invention

          The invention provides a novel class of immunosuppressant compounds useful in the treatment or prevention of diseases or disorders mediated by lymphocyte interactions, particularly diseases associated with EDG receptor mediated signal transduction.

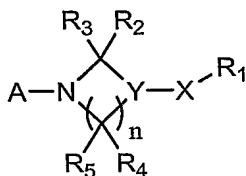
#### Background

15           EDG receptors belong to a family of closely related, lipid activated G-protein coupled receptors. EDG-1, EDG-3, EDG-5, EDG-6, and EDG-8 (also respectively termed S1P1, S1P3, S1P2, S1P4, and S1P5) are identified as receptors specific for sphingosine-1-phosphate (S1P). EDG2, EDG4, and EDG7 (also termed LPA1, LPA2, and LPA3, respectively) are receptors specific for lysophosphatidic (LPA). Among the S1P receptor  
20   isotypes, EDG-1, EDG-3 and EDG-5 are widely expressed in various tissues, whereas the expression of EDG-6 is confined largely to lymphoid tissues and platelets, and that of EDG-8 to the central nervous system. EDG receptors are responsible for signal transduction and are thought to play an important role in cell processes involving cell development, proliferation, maintenance, migration, differentiation, plasticity and apoptosis. Certain EDG  
25   receptors are associated with diseases mediated by lymphocyte interactions, for example, in transplantation rejection, autoimmune diseases, inflammatory diseases, infectious diseases and cancer. An alteration in EDG receptor activity contributes to the pathology and/or

symptomology of these diseases. Accordingly, molecules that themselves alter the activity of EDG receptors are useful as therapeutic agents in the treatment of such diseases.

## SUMMARY OF THE INVENTION

5 This application relates to compounds of Formula I:



in which:

n is 1, 2 or 3;

10 A is chosen from  $-X_1C(O)OR_6$ ,  $-X_1OP(O)(OR_6)_2$ ,  $-X_1P(O)(OR_6)_2$ ,  $-X_1S(O)_2OR_6$ ,  $-X_1P(O)(R_6)OR_6$  and 1*H*-tetrazol-5-yl; wherein  $X_1$  is chosen from a bond and  $C_{1-3}$ alkylene; and each  $R_6$  is independently chosen from hydrogen and  $C_{1-6}$ alkyl;

X is a bond or is chosen from  $C_{1-4}$ alkylene,  $-X_1OX_2-$ ,  $-X_1NR_7X_2-$ ,  $-X_1C(O)NR_7X_2-$ ,  $-X_1NR_7C(O)X_2-$ ,  $-X_1S(O)X_2-$ ,  $-X_1S(O)_2X_2-$ ,  $-X_1SX_2-$  and  $C_2$ -

15  $9$ heteroarylene; wherein  $X_1$  and  $X_2$  are independently chosen from a bond and  $C_{1-3}$ alkylene;  $R_7$  is chosen from hydrogen and  $C_{1-6}$ alkyl; and any heteroarylene of X is optionally substituted by a member of the group chosen from halo and  $C_{1-6}$ alkyl;

20 Y is chosen from  $C_{6-10}$ aryl and  $C_{2-9}$ heteroaryl, wherein any aryl or heteroaryl of Y can be optionally substituted with 1 to 3 radicals chosen from halo, hydroxy, cyano, nitro,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted  $C_{1-6}$ alkyl and halo-substituted  $C_{1-6}$ alkoxy;

25  $R_1$  is chosen from  $C_{6-10}$ aryl and  $C_{2-9}$ heteroaryl; wherein any aryl or heteroaryl of  $R_1$  is optionally substituted by a radical chosen from  $C_{6-10}$ aryl $C_{0-4}$ alkyl,  $C_{2-9}$ heteroaryl $C_{0-4}$ alkyl,  $C_{3-8}$ cycloalkyl $C_{0-4}$ alkyl,  $C_{3-8}$ heterocycloalkyl $C_{0-4}$ alkyl or  $C_{1-6}$ alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl group of  $R_1$  can be optionally substituted by one to five radicals chosen from halo,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted- $C_{1-6}$ alkyl and halo-substituted- $C_{1-6}$ alkoxy; and any alkyl group of  $R_1$  can optionally have a methylene replaced by an atom or group chosen from  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR_7-$  and  $-O-$ ; wherein  $R_7$  is chosen from hydrogen or  $C_{1-6}$ alkyl;

R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are independently chosen from hydrogen, C<sub>1-6</sub>alkyl, halo, hydroxy, C<sub>1-6</sub>alkoxy, halo-substituted C<sub>1-6</sub>alkyl and halo-substituted C<sub>1-6</sub>alkoxy; and the N-oxide derivatives, prodrug derivatives, protected derivatives, individual isomers and mixtures of isomers thereof; and the pharmaceutically acceptable salts and solvates (e.g. hydrates) of such compounds.

A second aspect of the invention is a pharmaceutical composition which contains a compound of Formula I or an N-oxide derivative, individual isomer or mixture of isomers thereof, or a pharmaceutically acceptable salt thereof, in admixture with one or more suitable excipients.

A third aspect of the invention is a method for treating a disease in an animal in which alteration of EDG receptor mediated signal transduction can prevent, inhibit or ameliorate the pathology and/or symptomology of the disease, which method comprises administering to the animal a therapeutically effective amount of a compound of Formula I or a N-oxide derivative, individual isomer or mixture of isomers thereof; or a pharmaceutically acceptable salt thereof.

A fourth aspect of the invention is the use of a compound of Formula I in the manufacture of a medicament for treating a disease in an animal in which alteration of EDG receptor mediated signal transduction contributes to the pathology and/or symptomology of the disease.

A fifth aspect of the invention is a process for preparing compounds of Formula I and the N-oxide derivatives, prodrug derivatives, protected derivatives, individual isomers and mixtures of isomers thereof; and the pharmaceutically acceptable salts thereof.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides compounds that are useful in the treatment and/or prevention of diseases or disorders mediated by lymphocyte interactions. Also provided are methods for treating such diseases or disorders.

### Definitions

In this specification, unless otherwise defined:

“Alkyl” as a group and as a structural element of other groups, for example halo-substituted-alkyl, alkoxy, acyl, alkylthio, alkylsulfonyl and alkylsulfinyl, can be either

straight-chained or branched. "Alkenyl" as a group and as a structural element of other groups contains one or more carbon-carbon double bonds, and can be either straight-chain, or branched. Any double bonds can be in the cis- or trans- configuration. "Alkynyl" as a group and as structural element of other groups and compounds contains at least one C  $\equiv$  C triple bond and can also contain one or more C=C double bonds, and can, so far as possible, be either straight-chain or branched. Any cycloalkyl group, alone or as a structural element of other groups can contain from 3 to 8 carbon atoms, preferably from 3 to 6 carbon atoms. "Alkylene" and "alkenylene" are divalent radicals derived from "alkyl" and "alkenyl" groups, respectively. In this application, any alkyl group of R<sup>1</sup> can be optionally interrupted by a member of the group selected from -S-, -S(O)-, -S(O)<sub>2</sub>-, -NR<sup>20</sup>- and -O- (wherein R<sup>20</sup> is hydrogen or C<sub>1-6</sub>alkyl). These groups include -CH<sub>2</sub>-O-CH<sub>2</sub>-, -CH<sub>2</sub>-S(O)<sub>2</sub>-CH<sub>2</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-NR<sup>20</sup>-CH<sub>2</sub>-, -CH<sub>2</sub>-O-(CH<sub>2</sub>)<sub>2</sub>-, and the like.

"Aryl" means a monocyclic or fused bicyclic aromatic ring assembly containing six to ten ring carbon atoms. For example, C<sub>6-12</sub>aryl can be phenyl, biphenyl or naphthyl, preferably phenyl. A fused bicyclic ring can be partially saturated, for example, 1,2,3,4-tetrahydro-naphthalene, and the like. "Arylene" means a divalent radical derived from an aryl group. For example, arylene as used in this application can be phenylene, biphenylene, naphthylene and the like.

"Halo" or "halogen" means F, Cl, Br or I, preferably F or Cl. Halo-substituted alkyl groups and compounds can be partially halogenated or perhalogenated, whereby in the case of multiple halogenation, the halogen substituents can be identical or different. A preferred perhalogenated alkyl group is for example trifluoromethyl or trifluoromethoxy.

"Heteroaryl" means aryl, as defined in this application, with the addition of at least one heteroatom moiety selected from N, O or S, and each ring is comprised of 5 to 6 ring atoms, unless otherwise stated. For example, C<sub>2</sub>heteroaryl includes oxadiazole, triazole, and the like. C<sub>9</sub>heteroaryl includes quinoline, 1,2,3,4-tetrahydro-quinoline, and the like. C<sub>2-9</sub>heteroaryl as used in this application includes thienyl, pyridinyl, furanyl, isoxazolyl, benzoxazolyl or benzo[1,3]dioxolyl, preferably thienyl, furanyl or pyridinyl.

"Heteroarylene" means heteroaryl, as defined in this application, provided that the ring assembly comprises a divalent radical. A fused bicyclic heteroaryl ring system can be

partially saturated, for example, 2,3-dihydro-1H-isoindole, 1,2,3,4-tetrahydro-quinoline, and the like.

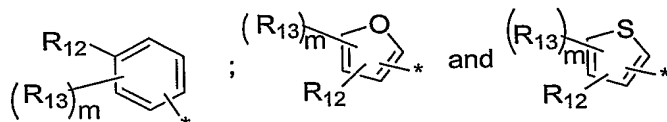
As used in the present invention, an EDG-1 selective compound (agent or modulator) has a specificity that is selective for EDG-1 over EDG-3 and over one or more of EDG-5, EDG-6, and EDG-8. As used herein, selectivity for one EDG receptor (a “selective receptor”) over another EDG receptor (a “non-selective receptor”) means that the compound has a much higher potency in inducing activities mediated by the selective EDG receptor (e.g., EDG-1) than that for the non-selective S1P-specific EDG receptor. If measured in a GTP- $\gamma$ S binding assay (as described in the Example below), an EDG-1 selective compound typically has an EC50 (effective concentration that causes 50% of the maximum response) for a selective receptor (EDG-1) that is at least 5, 10, 25, 50, 100, 500, or 1000 fold lower than its EC50 for a non-selective receptor (e.g., one or more of EDG-3, EDG-5, EDG-6, and EDG-8).

#### **Detailed Description of the Invention**

The invention provides compounds that are useful for treating or preventing diseases or disorders that are mediated by lymphocyte interactions. In one embodiment, for compounds of Formula I, R<sub>1</sub> is phenyl, naphthyl, furanyl or thienyl optionally substituted by C<sub>6-10</sub>arylC<sub>0-4</sub>alkyl, C<sub>2-9</sub>heteroarylC<sub>0-4</sub>alkyl, C<sub>3-8</sub>cycloalkylC<sub>0-4</sub>alkyl, C<sub>3-8</sub>heterocycloalkylC<sub>0-4</sub>alkyl or C<sub>1-6</sub>alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl group of R<sub>1</sub> can be optionally substituted by one to five radicals chosen from halo, C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkoxy, halo-substituted-C<sub>1-6</sub>alkyl and halo-substituted-C<sub>1-6</sub>alkoxy; and any alkyl group of R<sub>1</sub> can optionally have a methylene replaced by an atom or group chosen from -S-, -S(O)-, -S(O)<sub>2</sub>-, -NR<sub>7</sub>- and -O-; wherein R<sub>7</sub> is hydrogen or C<sub>1-6</sub>alkyl.

In another embodiment, Y is chosen from phenyl and benzooxazolyl; and X is a bond or is chosen from -X<sub>1</sub>OX<sub>2</sub>- and C<sub>4-6</sub>heteroarylene; wherein X<sub>1</sub> and X<sub>2</sub> are independently chosen from a bond and C<sub>1-3</sub>alkylene; wherein any heteroarylene of X is optionally substituted by a member of the group chosen from halo and C<sub>1-6</sub>alkyl.

In another embodiment, R<sub>1</sub> is chosen from:

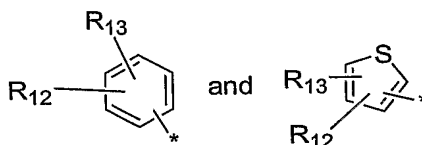


wherein the asterisk is the point of attachment of  $R_1$  with X; m is chosen from 1 and 2;  $R_{12}$  is selected from hydrogen,  $C_{6-10}$ aryl $C_{0-4}$ alkyl,  $C_{2-9}$ heteroaryl $C_{0-4}$ alkyl,  $C_{3-8}$ cycloalkyl $C_{0-4}$ alkyl,  $C_{3-8}$ heterocycloalkyl $C_{0-4}$ alkyl or  $C_{1-6}$ alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl group of  $R_{12}$  can be optionally substituted by one to three radicals chosen from halo,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted- $C_{1-6}$ alkyl and halo-substituted- $C_{1-6}$ alkoxy; and any alkyl group of  $R_{12}$  can optionally have a methylene replaced by an atom or group chosen from  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR_{10}-$  and  $-O-$ ; wherein  $R_{10}$  is hydrogen or  $C_{1-6}$ alkyl; and  $R_{13}$  is chosen from halo,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted- $C_{1-6}$ alkyl and halo-substituted- $C_{1-6}$ alkoxy.

In another embodiment, A is  $-(CH_2)_2C(O)OH$ ; and  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  are hydrogen.

In another embodiment, n is 1 or 2; Y is selected from phenyl and benzooxazolyl; and X is a bond or selected from [1,2,4]oxadiazole,  $-CH_2O-$ ,  $-OCH_2-$ , isoxazoles and [1,3,4]oxadiazole.

In another embodiment,  $R_1$  is selected from:



wherein  $R_{12}$  is selected from hydrogen, phenyl and cyclohexyl; wherein any phenyl or cyclohexyl of  $R_{12}$  is optionally substituted with methyl; and  $R_{13}$  is selected from trifluoromethyl, methyl and ethyl.

Preferred compounds of the invention are selected from 3-{6-[3-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-[6-(2-trifluoromethyl-biphenyl-4-yloxymethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-{5-[5-(2-trifluoromethyl-biphenyl-4-yl)-isoxazol-3-yl]-1,3-dihydro-isoindol-2-yl}-propionic acid, 3-{5-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-1,3-dihydro-isoindol-2-yl}-propionic acid, 3-{5-[5-(2-trifluoromethyl-biphenyl-4-yl)-

[1,3,4]oxadiazol-2-yl]-1,3-dihydro-isoindol-2-yl]-propionic acid, 3-[2-(2-trifluoromethyl-biphenyl-4-yl)-5,7-dihydro-oxazolo[4,5-f]isoindol-6-yl]-propionic acid, 3-{7-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-{6-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-{6-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,3,4]oxadiazol-2-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-[6-(3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(4-cyclohexyl-3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(2-trifluoromethyl-biphenyl-4-ylmethoxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[7-(4-cyclohexyl-3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(4-cyclohexyl-3-methyl-phenoxy-methyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(4-cyclohexyl-3-ethyl-phenoxy-methyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(2-ethyl-biphenyl-4-yloxy-methyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid and 3-[6-(2-ethyl-3'-methyl-biphenyl-4-yloxy-methyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid.

Further preferred compounds are also shown in the examples and table 1, *infra*.

The invention provides forms of the compound that have the hydroxyl or amine group present in a protected form; these function as prodrugs. Prodrugs are compounds that are converted into an active drug form after administration, through one or more chemical or biochemical transformations. Forms of the compounds of the present invention that are readily converted into the claimed compound under physiological conditions are prodrugs of the claimed compounds and are within the scope of the present invention. Examples of prodrugs include forms where a hydroxyl group is acylated to form a relatively labile ester such as an acetate ester, and forms where an amine group is acylated with the carboxylate group of glycine or an L-amino acid such as serine, forming an amide bond that is particularly susceptible to hydrolysis by common metabolic enzymes.

Compounds of Formula I can exist in free form or in salt form, e.g. addition salts with inorganic or organic acids. Where hydroxyl groups are present, these groups can also be present in salt form, e.g. an ammonium salt or salts with metals such as lithium, sodium, potassium, calcium, zinc or magnesium, or a mixture thereof. Compounds of Formula I and their salts in hydrate or solvate form are also part of the invention.



When the compounds of Formula I have asymmetric centers in the molecule, various optical isomers are obtained. The present invention also encompasses enantiomers, racemates, diastereoisomers and mixtures thereof. Moreover, when the compounds of Formula I include geometric isomers, the present invention embraces cis-compounds, trans-  
5 compounds and mixtures thereof. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms or unsaturated bonds as mentioned above.

***Methods and Pharmaceutical Compositions for Treating Immunomodulatory Conditions***

The compounds of Formula I in free form or in pharmaceutically acceptable salt  
10 form, exhibit valuable pharmacological properties, e.g. lymphocyte recirculation modulating properties, for example, as indicated by the *in vitro* and *in vivo* tests of Example 3 and are therefore indicated for therapy. Compounds of Formula I preferably show an EC<sub>50</sub> in the range of  $1 \times 10^{-11}$  to  $1 \times 10^{-5}$  M, preferably less than 50nM. The compounds exhibit selectivity for one or more EDG/S1P receptors, preferably EDG-1/S1P-1. EDG-1/S1P-1  
15 selective modulators of the present invention can be identified by assaying a compound's binding to EDG-1/S1P-1 and one or more of the other EDG/S1P receptors (e.g., EDG-3/S1P-3, EDG-5/S1P-2, EDG-6/S1P-4, and EDG-8/S1P-5). An EDG-1/S1P-1 selective modulator usually has an EC<sub>50</sub> for the EDG-1/S1P-1 receptor in the range of  $1 \times 10^{-10}$  to  $1 \times 10^{-5}$  M, preferably less than 50 nM, more preferably less than 5 nM. It also has an EC<sub>50</sub>  
20 for one or more of the other EDG/S1P receptors that is at least 5, 10, 25, 50, 100, 500, or 1000 fold higher than its EC<sub>50</sub> for EDG-1/S1P-1. Thus, some of the EDG-1/S1P-1 modulatory compounds will have an EC<sub>50</sub> for EDG-1/S1P-1 that is less than 5 nM while their EC<sub>50</sub> for one or more of the other EDG/S1P receptors are at least 100 nM or higher. Other than assaying binding activity to the EDG/S1P receptors, EDG-1/S1P-1 selective  
25 agents can also be identified by examining a test agent's ability to modify a cellular process or activity mediated by an EDG/S1P receptor.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by lymphocytes interactions, for example in transplantation, such as acute or chronic rejection of cell, tissue or organ allo- or xenografts  
30 or delayed graft function, graft versus host disease, autoimmune diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus, hashimoto's thyroiditis, multiple sclerosis,

myasthenia gravis, diabetes type I or II and the disorders associated therewith, vasculitis, pernicious anemia, Sjogren syndrome, uveitis, psoriasis, Graves ophthalmopathy, alopecia areata and others, allergic diseases, e.g. allergic asthma, atopic dermatitis, allergic rhinitis/conjunctivitis, allergic contact dermatitis, inflammatory diseases optionally with  
5 underlying aberrant reactions, e.g. inflammatory bowel disease, Crohn's disease or ulcerative colitis, intrinsic asthma, inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, atherosclerosis, osteoarthritis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, cutaneous manifestations of immunologically-mediated disorders, inflammatory eye disease, keratoconjunctivitis,  
10 myocarditis or hepatitis, ischemia/reperfusion injury, e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, traumatic shock, T cell lymphomas or T cell leukemias, infectious diseases, e.g. toxic shock (e.g. superantigen induced), septic shock, adult respiratory distress syndrome or viral infections, e.g. AIDS, viral hepatitis, chronic bacterial infection, or senile dementia. Examples of cell, tissue or solid organ transplants  
15 include e.g. pancreatic islets, stem cells, bone marrow, corneal tissue, neuronal tissue, heart, lung, combined heart-lung, kidney, liver, bowel, pancreas, trachea or oesophagus. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired.

Furthermore, the compounds of formula I are useful in cancer chemotherapy,  
20 particularly for cancer chemotherapy of solid tumors, e.g. breast cancer, or as an anti-angiogenic agent.

The required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 2.5 mg/kg per  
25 body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 100 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 1 to 50 mg active ingredient.

The compounds of Formula I can be administered by any conventional route, in  
30 particular enterally, for example, orally, e.g. in the form of tablets or capsules, or parenterally, for example, in the form of injectable solutions or suspensions, topically, e.g. in

the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of Formula I in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable carrier or diluent can be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent.

The compounds of Formula I can be administered in free form or in pharmaceutically acceptable salt form, for example, as indicated above. Such salts can be prepared in a conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by lymphocytes, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.3 A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

1.4 A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 to 1.4 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 to 1.4 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use  
5 in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 to 1.4 above.

The compounds of formula I may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, other drugs e.g. immunosuppressive or immunomodulating agents or other anti-inflammatory agents, e.g. for the treatment or  
10 prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders, or a chemotherapeutic agent, e.g. a malignant cell anti-proliferative agent. For example the compounds of formula I may be used in combination with a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578 or AP23573; an ascomycin having  
15 immunosuppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g. monoclonal antibodies to leukocyte receptors, e.g. MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28,  
20 CD40, CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y ; adhesion molecule inhibitors, e.g. LFA-1  
25 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a chemotherapeutic agent.

By the term "chemotherapeutic agent" is meant any chemotherapeutic agent and it includes but is not limited to,

- i. an aromatase inhibitor,
- 30 ii. an anti-estrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,

- iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
- iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
- v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,
- vi. a bradykinin 1 receptor or an angiotensin II antagonist,
- vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon  $\alpha$ , an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
- viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,
- ix. a telomerase inhibitor, e.g. telomestatin,
- x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a proteasome inhibitor, e.g. PS-341, and/or
- xi. a mTOR inhibitor.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol, respectively. The term includes, but is not limited to steroids, especially atamestane, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "anti-estrogen" as used herein relates to a compound that antagonizes the effect of estrogens at the estrogen receptor level. The term includes, but is not limited to tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. A combination of the invention comprising a chemotherapeutic agent which is an anti-estrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term “anti-androgen” as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, but is not limited to, bicalutamide.

5 The term “gonadorelin agonist” as used herein includes, but is not limited to abarelix, goserelin and goserelin acetate.

The term “topoisomerase I inhibitor” as used herein includes, but is not limited to topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804).

10 The term “topoisomerase II inhibitor” as used herein includes, but is not limited to the anthracyclines such as doxorubicin, daunorubicin, epirubicin, idarubicin and nemorubicin, the anthraquinones mitoxantrone and losoxantrone, and the podophyllotoxines etoposide and teniposide.

The term “microtubule active agent” relates to microtubule stabilizing and microtubule destabilizing agents including, but not limited to taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g., vinblastine, especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof.

20 The term “alkylating agent” as used herein includes, but is not limited to busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel<sup>TM</sup>).

The term “antineoplastic antimetabolite” includes, but is not limited to 5-fluorouracil, capecitabine, gemcitabine, cytarabine, fludarabine, thioguanine, methotrexate and edatrexate.

25 The term “platin compound” as used herein includes, but is not limited to carboplatin, cis-platin and oxaliplatin.

The term “compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds” as used herein includes, but is not limited to protein tyrosine kinase and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases

30

(VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-  
5 fusion products (e.g. BCR-Abl), members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

10 Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof,  
15 e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad.  
20 Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin<sup>TM</sup>, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin<sup>TM</sup>, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies  
25 or anti-VEGF receptor antibodies, e.g. RhuMab.

By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

Compounds which target, decrease or inhibit the activity of the epidermal growth  
30 factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and

ErbB4 or bind to EGF or EGF related ligands, or which have a dual inhibiting effect on the ErbB and VEGF receptor kinase and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180) or PCT/EP02/08780; e.g. trastuzumab (Herpetin<sup>R</sup>), cetuximab, Iressa, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

Compounds which target, decrease or inhibit the activity of c-AbI family members and their gene fusion products are, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296 110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safinol, BAY 43-9006, Bryostatin 1, Perifosine; Ilmofofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are, e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

Compounds which induce cell differentiation processes are, e.g. retinoic acid,  $\alpha$ -,  $\gamma$ - or  $\delta$ -tocopherol or  $\alpha$ -,  $\gamma$ - or  $\delta$ -tocotrienol.



The term cyclooxygenase inhibitor as used herein includes, but is not limited to, e.g. celecoxib (Celebrex<sup>R</sup>), rofecoxib (Vioxx<sup>R</sup>), etoricoxib, valdecoxib or a 5-alkyl-2-arylamino phenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

5 The term "histone deacetylase inhibitor" as used herein includes, but is not limited to MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

The term "bisphosphonates" as used herein includes, but is not limited to, etridronic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid.

10 The term "matrix metalloproteinase inhibitor" as used herein includes, but is not limited to collagen peptidomimetic and non-peptidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

15 The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof, e.g. 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-O-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or  
20 rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573.

25 Where the compounds of formula I are administered in conjunction with other immunosuppressive / immunomodulatory, anti-inflammatory or chemotherapeutic therapy, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth.

In accordance with the foregoing the present invention provides in a yet further aspect:

30 5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a compound of formula I and

at least a second drug substance, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a compound of formula I as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as disclosed above. The kit may comprise instructions for its administration.

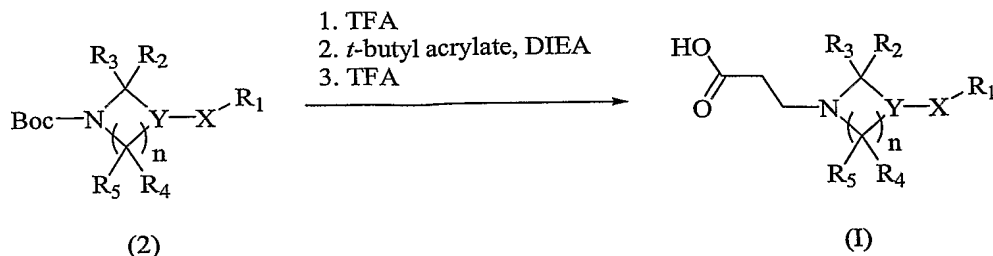
The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the 2 compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

### ***Methods for Preparing Compounds of the Invention***

The present invention also includes processes for the preparation of immunomodulatory compounds of the invention. In the reactions described, it can be necessary to protect reactive functional groups, for example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups can be used in accordance with standard practice, for example, see T.W. Greene and P. G. M. Wuts in "Protective Groups in Organic Chemistry", John Wiley and Sons, 1991.

Compounds of Formula I can be prepared by proceeding as in the following reaction scheme:



in which  $n$ ,  $X$ ,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  are as defined for Formula I above.

Compounds of Formula I can be prepared sequentially by treating a compound of formula 2 with a suitable acid (e.g. TFA, and the like), reacting with *t*-butyl acrylate in the presence of a suitable amine (e.g. DIEA, and the like) and removing the *t*-butyl protecting group with a suitable acid (e.g. TFA, and the like). The reaction proceeds at a temperature of about 0 to about 120°C and can take up to about 24 hours to complete.

#### ***Additional Processes for Preparing Compounds of the Invention:***

A compound of the invention can be prepared as a pharmaceutically acceptable acid addition salt by reacting the free base form of the compound with a pharmaceutically acceptable inorganic or organic acid. Alternatively, a pharmaceutically acceptable base addition salt of a compound of the invention can be prepared by reacting the free acid form of the compound with a pharmaceutically acceptable inorganic or organic base. Alternatively, the salt forms of the compounds of the invention can be prepared using salts of the starting materials or intermediates.

The free acid or free base forms of the compounds of the invention can be prepared from the corresponding base addition salt or acid addition salt form, respectively. For example a compound of the invention in an acid addition salt form can be converted to the corresponding free base by treating with a suitable base (e.g., ammonium hydroxide solution, sodium hydroxide, and the like). A compound of the invention in a base addition salt form can be converted to the corresponding free acid by treating with a suitable acid (e.g., hydrochloric acid, etc.).

Compounds of the invention in unoxidized form can be prepared from N-oxides of compounds of the invention by treating with a reducing agent (e.g., sulfur, sulfur dioxide, triphenyl phosphine, lithium borohydride, sodium borohydride, phosphorus trichloride, tribromide, or the like) in a suitable inert organic solvent (e.g. acetonitrile, ethanol, aqueous dioxane, or the like) at 0 to 80°C.

Prodrug derivatives of the compounds of the invention can be prepared by methods known to those of ordinary skill in the art (e.g., for further details see Saulnier et al., (1994), *Bioorganic and Medicinal Chemistry Letters*, Vol. 4, p. 1985). For example, appropriate prodrugs can be prepared by reacting a non-derivatized compound of the invention with a suitable carbamylating agent (e.g., 1,1-acyloxyalkylcarbanochloridate, para-nitrophenyl carbonate, or the like).

Protected derivatives of the compounds of the invention can be made by means known to those of ordinary skill in the art. A detailed description of techniques applicable to the creation of protecting groups and their removal can be found in T W. Greene, "Protecting Groups in Organic Chemistry", 3<sup>rd</sup> edition, John Wiley and Sons, Inc., 1999.

Compounds of the present invention can be conveniently prepared, or formed during the process of the invention, as solvates (e.g., hydrates). Hydrates of compounds of the present invention can be conveniently prepared by recrystallization from an aqueous/organic solvent mixture, using organic solvents such as dioxin, tetrahydrofuran or methanol.

Compounds of the invention can be prepared as their individual stereoisomers by reacting a racemic mixture of the compound with an optically active resolving agent to form a pair of diastereoisomeric compounds, separating the diastereomers and recovering the optically pure enantiomers. While resolution of enantiomers can be carried out using covalent diastereomeric derivatives of the compounds of the invention, dissociable complexes are preferred (e.g., crystalline diastereomeric salts). Diastereomers have distinct physical properties (e.g., melting points, boiling points, solubilities, reactivity, etc.) and can be readily separated by taking advantage of these dissimilarities. The diastereomers can be separated by chromatography, or preferable, by separation/resolution techniques based upon differences in solubility. The optically pure enantiomer is then recovered, along with the resolving agent, by any practical means that would not result in racemization. A more

detailed description of the techniques applicable to the resolution of stereoisomers of compounds from their racemic mixture can be found in Jean Jacques, Andre Collet, Samuel H. Wilen, "Enantiomers, Racemates and Resolutions", John Wiley And Sons, Inc., 1981.

5           In summary, the compounds of Formula I can be made by a process, which involves:

- (a) the above reaction scheme; and
- (b) optionally converting a compound of the invention into a pharmaceutically acceptable salt;
- 10           (c) optionally converting a salt form of a compound of the invention to a non-salt form;
- (d) optionally converting an unoxidized form of a compound of the invention into a pharmaceutically acceptable N-oxide;
- (e) optionally converting an N-oxide form of a compound of the invention to its
- 15           unoxidized form;
- (f) optionally resolving an individual isomer of a compound of the invention from a mixture of isomers;
- (g) optionally converting a non-derivatized compound of the invention into a pharmaceutically acceptable prodrug derivative; and
- 20           (h) optionally converting a prodrug derivative of a compound of the invention to its non-derivatized form.

Insofar as the production of the starting materials is not particularly described, the compounds are known or can be prepared analogously to methods known in the art or as disclosed in the Examples hereinafter.

25           One of skill in the art will appreciate that the above transformations are only representative of methods for preparation of the compounds of the present invention, and that other well known methods can similarly be used.

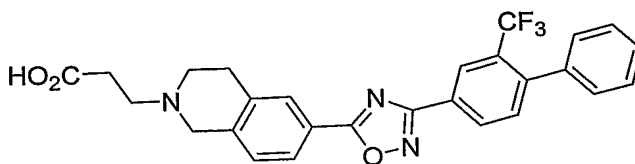
## EXAMPLES

The following examples provide detailed descriptions of the preparation of representative compounds and are offered to illustrate, but not to limit the present invention.

5

## Example 1

Synthesis of 3-{6-[3-(2-Trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid



10

To a suspension of 3,4-dihydro-1H-isoquinoline-2,6-dicarboxylic acid 2-tert-butyl ester (96 mg, 0.348 mmol) in toluene (4 mL) is added SOCl<sub>2</sub> (254 μL, 10 eq.). The mixture is heated to reflux for 3 hours. All the solvent is removed under reduced pressure. The residue is redissolved in toluene and evaporated to dryness twice to remove excess HCl and is dried under high vacuum for 2 hours to give crude 6-chlorocarbonyl-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester .

15

20

In a separate flask is charged with N-hydroxy-2-trifluoromethyl-biphenyl-4-carboxamidine (82 mg, 0.29 mmol) and DIEA (242 μL, 4 eq.) and CH<sub>2</sub>Cl<sub>2</sub> (5 mL). The mixture is cooled to 0°C using an ice-salt bath. The chloride from the previous step is dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and added slowly. After addition, the resulting mixture is warmed to room temperature and is stirred for 2 hours. All the solvent is evaporated and the mixture is purified by column chromatography (silica gel, EtOAc/Hexane, gradient) to 120 mg of the desired product. The product is dissolved in THF (2 mL) and mixed with TBAF (444 μL, 2 eq.) in a microwave vial. The mixture is heated to 100°C for 15 minutes using microwave irradiation. All the solvent is evaporated and the residue is purified by column chromatography (silica gel, EtOAc/Hexane, gradient) to give 70 mg of 6-[3-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester.

25

30

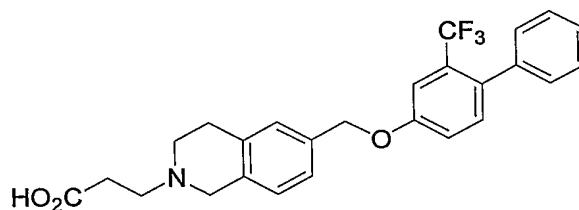
To a solution of 6-[3-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester (68 mg, 0.13 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1

mL) is added TFA(2 mL). The mixture is stirred at room temperature for 30 minutes. All the solvents are removed under reduced pressure. The mixture is dissolved in CH<sub>3</sub>OH (1.5 mL). Then DIEA (119  $\mu$ L, 10 eq.) and acrylic acid tert-butyl ester (38  $\mu$ L, 2 eq.) are added. The mixture is heated to 90°C for 20 minutes using microwave irradiation. All the solvent is evaporated and the crude product of 3-{6-[3-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid tert-butyl ester is used in the next step without further purification.

To a solution of crude 3-{6-[3-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid tert-butyl ester in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) is added TFA(1 mL). The mixture is stirred at room temperature for an hour. All the solvents are evaporated. The mixture is purified by reverse phase preparative LC/MS to give 3-{6-[3-(2-Trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid: <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.52 (s, 1H), 8.38 (d, 1H), 8.15 (s, 1H), 8.10 (d, 1H), 7.68 (d, 1H), 7.50 (m, 4H), 7.38 (m, 2H), 3.20-3.50 (m, 8H), 2.92 (t, 2H), MS (ES<sup>+</sup>): (494.10, M+1)<sup>+</sup>.

### Example 2

#### Synthesis of 3-[6-(2-Trifluoromethyl-biphenyl-4-yloxymethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid



To a solution of 6-hydroxymethyl-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester (85 mg, 0.323 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) is added a solution of 4-chloro-3-trifluoromethyl-phenol (76 mg, 1.23 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL), PPh<sub>3</sub> (127 mg, 1.5 eq.), and 1,1'-(azodicarbonyl)-dipiperidine (122 mg, 1.5 eq.). The mixture is stirred at room temperature overnight. All the solvent is removed under reduced pressure and the mixture is purified by column chromatography (silica gel, EtOAc/Hexane, gradient) to give 70 mg of 6-

(4-chloro-3-trifluoromethyl-phenoxyethyl)-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester

To a solution of 6-(4-chloro-3-trifluoromethyl-phenoxyethyl)-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester (70 mg, 0.158 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) is added TFA (1 mL). The mixture is stirred at room temperature for 30 minutes. All the solvents are removed under reduced pressure. The mixture is dissolved in CH<sub>3</sub>OH (1.5 mL). Then DIEA (269  $\mu$ L, 10 eq.) and acrylic acid tert-butyl ester (46  $\mu$ L, 2 eq.) are added. The mixture is heated to 90°C for 20 minutes using microwave irradiation. All the solvent is evaporated and the mixture is purified by column chromatography (silica gel, EtOAc/Hexane, gradient) to give 56 mg of 3-[6-(4-chloro-3-trifluoromethyl-phenoxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid tert-butyl ester.

A microwave vial is charged with 3-[6-(4-chloro-3-trifluoromethyl-phenoxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid tert-butyl ester (56 mg, 0.119 mmol), phenylboronic acid (17 mg, 1.2 eq.), Pd(OAc)<sub>2</sub> (1.3 mg, 5 mol%), (dicyclohexylphosphino)biphenyl (4.2mg, 10 mol%), KF (21mg, 3 eq.), and THF (0.25 mL). The resulting mixture is heated to 120°C using microwave irradiation for 20 minutes. The mixture is filtered through celite. The celite is washed with EtOAc several times. The filtrate is then concentrated to give a dark oil. The mixture is purified by column chromatography (silica gel, EtOAc/Hexane, gradient) to give 3-[6-(2-trifluoromethyl-biphenyl-4-yloxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid tert-butyl ester.

To a solution of 3-[6-(2-trifluoromethyl-biphenyl-4-yloxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid tert-butyl ester (35 mg, 0.068 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) is added TFA (1 mL). The mixture is stirred at room temperature for an hour. All the solvents are evaporated. The mixture is purified by reverse phase preparative LC/MS to give 3-[6-(2-trifluoromethyl-biphenyl-4-yloxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid: <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  7.45 (m, 2H), 7.38-7.41 (m, 3H), 7.35 (s, 1H), 7.16-7.23 (m, 5H), 4.85 (s, 2H), 4.54 (s, 2H), 3.68 (m, 2H), 3.60 (t, 2H), 3.27 (t, 2H), 2.96 (t, 2H), MS (ES<sup>+</sup>): (456.20, M+1)<sup>+</sup>.



By repeating the procedure described in the above examples, using appropriate starting materials, the following compounds of Formula I are obtained as identified in Table 1.

TABLE 1

Compound	Structure	Physical Data MS ES (M+1)
1		479.2
2		480.2
3		480.2
4		453.1
5		494.2
6		494.2
7		494.2
8		380.1
9		462.2

Compound	Structure	Physical Data MS ES (M+1)
10		456.2
11		456.2
12		462.3
13		
14		
15		
16		

### Example 3

#### Compounds of Formula I Exhibit Biological Activity

**A. In vitro: GPCR activation assay measuring GTP [ $\gamma$ - $^{35}$ S] binding to membranes prepared from CHO cells expressing human EDG receptors**

EDG-1 (S1P<sub>1</sub>) GTP [ $\gamma$ - $^{35}$ S] binding assay: Homogenized membranes are prepared from CHO cell clones stably expressing a human EDG-1 N-terminal c-myc tag. Cells are grown in suspension in two 850 cm<sup>2</sup> roller bottles for three or four days before harvesting. The cells are centrifuged down, washed once with cold PBS, and resuspended in  $\leq$ 20 ml of Buffer A (20 mM HEPES, pH 7.4, 10 mM EDTA, EDTA-free complete protease inhibitor cocktail [1 tablet/25 ml]). The cell suspension is homogenized on ice, using a Polytron homogenizer at 30000 rpm at three intervals of 15 seconds each. The homogenate is first centrifuged at 2000 rpm on a tabletop low speed centrifuge for 10 minutes. The supernatant, after passing through a cell strainer, is then re-centrifuged at 50,000 x g for 25 minutes at 4°C. The pellet is resuspended into buffer B (15% glycerol, 20 mM HEPES, pH 7.4, 0.1 mM EDTA, EDTA-free complete protease inhibitor cocktail [1 tablet/10 ml]). Protein concentration of the prep is determined using the BCA Protein Assay kit (Pierce) using BSA as standard. The membranes are aliquoted and kept frozen at -80°C.

Solutions of test compounds ranging from 10mM to 0.01nM are prepared in DMSO. S1P is diluted in 4% BSA solution as positive controls. The desired amount of membrane prep is diluted with ice-cold assay buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.1% Fatty acid-free BSA, 5  $\mu$ M GDP) and vortexed well. 2  $\mu$ l or less of compound is distributed into each well of a round-bottom 96-well polystyrene assay plate, followed by addition of 100  $\mu$ l of diluted membranes (3-10  $\mu$ g/well) and kept on ice until the addition of hot GTP $\gamma$ S. [ $^{35}$ S]-GTP $\gamma$ S is diluted 1:1000 (v/v) with cold assay buffer and 100  $\mu$ l is added into each well. The reaction is carried out at room temperature for 90 minutes before the membranes are harvested onto Perkin-Elmer Unifilter<sup>®</sup> GF/B-96 filter plate using a Packard Filtermate Harvester. After several washes with wash buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl<sub>2</sub>), and a rinse with 95% ethanol, the filter is dried in a 37°C oven for 30 minutes. MicroScint-20 is added and the plate sealed for scintillation counting on TopCount. EC50 values are obtained by fitting the GTP [ $\gamma$ - $^{35}$ S] binding curves (raw data) with the dose response curve-fitting tool of GraphPad Prism. Six or twelve different concentrations are used to generate a concentration response curve (using three data points per concentration).

EDG-3,-5,-6 and -8 GTP [ $\gamma$ - $^{35}$ S] binding assays are carried out in a comparable manner to the EDG-1 GTP [ $\gamma$ - $^{35}$ S] binding assay using membranes from CHO cells stably expressing c-terminal c-myc tagged or untagged receptors. For each membrane preparation, titration experiments are first run with S1P control to determine the optimal amount of membranes to be added per assay well. Compounds of the invention were tested according to the above assay and were observed to exhibit selectivity for the EDG-1 receptor. For example, 3-[6-(4-cyclohexyl-3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid (compound 9) has an EC<sub>50</sub> of 0.2 nM in the above assay and is at least 1000 fold selective for EDG-1 compared to one or more of the other receptors including EDG-3, EDG-5, EDG-6 and EDG-8.

**B. In vitro: FLIPR calcium flux assay**

Compounds of the invention are tested for agonist activity on EDG-1, EDG-3, EDG-5, and EDG-6 with a FLIPR calcium flux assay. Briefly, CHO cells expressing an EDG receptor are maintained in F-12K medium (ATCC), containing 5% FBS, with 500ug/ml of G418.

Prior to the assay, the cells are plated in 384 black clear bottom plates at the density of 10,000 cells/well/25 $\mu$ l in the medium of F-12K containing 1% FBS. The second day, the cells are washed three times (25  $\mu$ l/each) with washing buffer. About 25  $\mu$ l of dye are added to each well and incubated for 1 hour at 37°C and 5% CO<sub>2</sub>. The cells are then washed four times with washing buffer (25  $\mu$ l/each). The calcium flux is assayed after adding 25  $\mu$ l of SEQ2871 solution to each well of cells. The same assay is performed with cells expressing each of the different EDG receptors. Titration in the FLIPR calcium flux assay is recorded over a 3-minute interval, and quantitated as maximal peak height percentage response relative to EDG-1 activation.

**C. In vivo: Screening Assays for measurement of blood lymphocyte depletion and assessment of heart effect**

Measurement of circulating lymphocytes: Compounds are dissolved in DMSO and diluted to obtain a final concentration of 4% DMSO (v/v, final concentration) and then further diluted in a constant volume of Tween80 25%/H<sub>2</sub>O, v/v. Tween80 25%/H<sub>2</sub>O (200  $\mu$ l), 4% DMSO, and FTY720 (10 $\mu$ g) are included as negative and positive controls,

respectively. Mice (C57bl/6 male, 6-10 week-old) are administered 250-300  $\mu$ L of compound solution orally by gavages under short isoflurane anesthesia.

Blood is collected from the retro-orbital sinus 6 and 24 hours after drug administration under short isoflurane anesthesia. Whole blood samples are subjected to hematology analysis. Peripheral lymphocyte counts are determined using an automated analyzer. Subpopulations of peripheral blood lymphocytes are stained by fluorochrome-conjugated specific antibodies and analyzed using a fluorescent activating cell sorter (Facsalibur). Two mice are used to assess the lymphocyte depletion activity of each compound screened. The result is an ED<sub>50</sub>, which is defined as the effective dose required displaying 50 % of blood lymphocyte depletion. Compounds of the invention were tested according to the above assay and were preferably found to exhibit an ED<sub>50</sub> of less than 1mg/kg, more preferably an ED<sub>50</sub> of less than 0.5 mg/kg. For example, 3-[6-(4-cyclohexyl-3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid (compound 9) exhibits an ED<sub>50</sub> of 0.2 mg/kg.

Assessment of Heart Effect: The effects of compounds on cardiac function are monitored using the AnonymOUSE ECG screening system. Electrocardiograms are recorded in conscious mice (C57bl/6 male, 6-10 week-old) before and after compound administration. ECG signals are then processed and analyzed using the e-MOUSE software. 90  $\mu$ g of compound further diluted in 200 $\mu$ l water, 15% DMSO are injected IP. Four mice are used to assess the heart effect of each compound.

**D: In vivo: Anti-angiogenic Activity**

Porous chambers containing (i) sphingosine-1-phosphate (5  $\mu$ M/chamber) or (ii) human VEGF (1  $\mu$ g/chamber) in 0.5 ml of 0.8% w/v agar (containing heparin, 20 U/ml) are implanted subcutaneously in the flank of mice. S1P or VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and blood content of the tissue. Mice are treated once a day orally or intravenously with a compound of formula I starting 4-6 hours before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 hours after the last dose. The weight and blood

content of the vascularized tissues around the chamber is determined. Animals treated with a compound of formula I show reduced weight and/or blood content of the vascularized tissues compared to animals treated with vehicle alone. Compounds of Formula I are anti-angiogenic when administered at a dose of about 0.3 to about 3mg/kg.

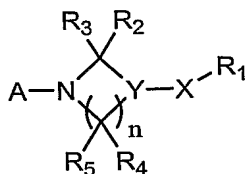
5    **E:    In vitro: Antitumor Activity**

A mouse breast cancer cell line originally isolated from mammary carcinomas is used, e.g. JygMC(A). The cell number is adjusted to  $5 \times 10^5$  for plating in fresh medium before the procedure. Cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 hours and then washed twice with PBS, followed by addition of fresh  
10    medium with 10% FCS and additionally incubated for another 12 hours. Thereafter the cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 hours. To release the cells from the block, the cells are washed twice with PBS and replated in fresh medium with 10% FCS. After synchronization, the cells are incubated with or without various concentrations of a compound of formula I for 3, 6, 9, 12, 18 or 24 hours. The cells  
15    are harvested after treatment with 0.2% EDTA, fixed with ice-cold 70% ethanol solution, hydrolyzed with 250  $\mu$ g/ml of RNaseA (type 1-A: Sigma Chem. Co.) at 37°C for 30 minutes and stained with propidium iodide at 10mg/ml for 20 minutes. After the incubation period, the number of cells is determined both by counting cells in a Coulter counter and by the SRB colorimetric assay. Under these conditions compounds of formula I inhibit the proliferation  
20    of the tumor cells at concentrations ranging from  $10^{-12}$  to  $10^{-6}$  M.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and  
25    understanding of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

## WE CLAIM

1. A compound of Formula I:



in which:

n is 1, 2 or 3;

A is chosen from  $-X_1C(O)OR_6$ ,  $-X_1OP(O)(OR_6)_2$ ,  $-X_1P(O)(OR_6)_2$ ,  $-X_1S(O)_2OR_6$ ,  $-X_1P(O)(R_6)OR_6$  and 1*H*-tetrazol-5-yl; wherein  $X_1$  is chosen from a bond and  $C_{1-3}$ alkylene; and each  $R_6$  is independently chosen from hydrogen and  $C_{1-6}$ alkyl;

X is a bond or is chosen from  $C_{1-4}$ alkylene,  $-X_1OX_2-$ ,  $-X_1NR_7X_2-$ ,  $-X_1C(O)NR_7X_2-$ ,  $-X_1NR_7C(O)X_2-$ ,  $-X_1S(O)X_2-$ ,  $-X_1S(O)_2X_2-$ ,  $-X_1SX_2-$  and  $C_{2-9}$ heteroarylene; wherein  $X_1$  and  $X_2$  are independently chosen from a bond and  $C_{1-3}$ alkylene;  $R_7$  is chosen from hydrogen and  $C_{1-6}$ alkyl; and any heteroarylene of X is optionally substituted by a member of the group chosen from halo and  $C_{1-6}$ alkyl;

Y is chosen from  $C_{6-10}$ aryl and  $C_{2-9}$ heteroaryl, wherein any aryl or heteroaryl of Y can be optionally substituted with 1 to 3 radicals chosen from halo, hydroxy, cyano, nitro,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted  $C_{1-6}$ alkyl and halo-substituted  $C_{1-6}$ alkoxy;

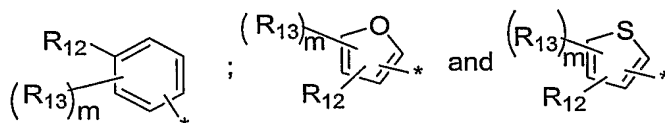
$R_1$  is chosen from  $C_{6-10}$ aryl and  $C_{2-9}$ heteroaryl; wherein any aryl or heteroaryl of  $R_1$  is optionally substituted by a radical chosen from  $C_{6-10}$ aryl $C_{0-4}$ alkyl,  $C_{2-9}$ heteroaryl $C_{0-4}$ alkyl,  $C_{3-8}$ cycloalkyl $C_{0-4}$ alkyl,  $C_{3-8}$ heterocycloalkyl $C_{0-4}$ alkyl or  $C_{1-6}$ alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl group of  $R_1$  can be optionally substituted by one to five radicals chosen from halo,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted- $C_{1-6}$ alkyl and halo-substituted- $C_{1-6}$ alkoxy; and any alkyl group of  $R_1$  can optionally have a methylene replaced by an atom or group chosen from  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR_7-$  and  $-O-$ ; wherein  $R_7$  is chosen from hydrogen or  $C_{1-6}$ alkyl;

$R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  are independently chosen from hydrogen,  $C_{1-6}$ alkyl, halo, hydroxy,  $C_{1-6}$ alkoxy, halo-substituted  $C_{1-6}$ alkyl and halo-substituted  $C_{1-6}$ alkoxy; and the pharmaceutically acceptable salts, hydrates, solvates, isomers and prodrugs thereof.

5           2. The compound of claim 1 in which  $R_1$  is phenyl, naphthyl, furanyl or thienyl optionally substituted by  $C_{6-10}$ aryl $C_{0-4}$ alkyl,  $C_{2-9}$ heteroaryl $C_{0-4}$ alkyl,  $C_{3-8}$ cycloalkyl $C_{0-4}$ alkyl,  $C_{3-8}$ heterocycloalkyl $C_{0-4}$ alkyl or  $C_{1-6}$ alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl group of  $R_1$  can be optionally substituted by one to five radicals chosen from halo,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted- $C_{1-6}$ alkyl and halo-substituted- $C_{1-6}$ alkoxy; and any alkyl group of  $R_1$  can optionally have a methylene replaced by an atom or group  
10           chosen from  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR_7-$  and  $-O-$ ; wherein  $R_7$  is hydrogen or  $C_{1-6}$ alkyl.

3.       The compound of claim 1 in which Y is chosen from phenyl and benzooxazolyl; and X is a bond or is chosen from  $-X_1OX_2-$  and  $C_{4-6}$ heteroarylene; wherein  
15        $X_1$  and  $X_2$  are independently chosen from a bond and  $C_{1-3}$ alkylene; wherein any heteroarylene of X is optionally substituted by a member of the group chosen from halo and  $C_{1-6}$ alkyl.

20       4.       The compound of claim 1 in which  $R_1$  is chosen from:



wherein the asterisk is the point of attachment of  $R_1$  with X; m is chosen from 1 and 2;  $R_{12}$  is selected from hydrogen,  $C_{6-10}$ aryl $C_{0-4}$ alkyl,  $C_{2-9}$ heteroaryl $C_{0-4}$ alkyl,  $C_{3-8}$ cycloalkyl $C_{0-4}$ alkyl,  $C_{3-8}$ heterocycloalkyl $C_{0-4}$ alkyl or  $C_{1-6}$ alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl group of  $R_{12}$  can be optionally substituted by one to three radicals chosen from halo,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkoxy, halo-substituted- $C_{1-6}$ alkyl and halo-substituted- $C_{1-6}$ alkoxy; and any alkyl group of  $R_{12}$  can optionally have a methylene replaced by an atom or group chosen from  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR_{10}-$  and  $-O-$ ; wherein  $R_{10}$  is  
25



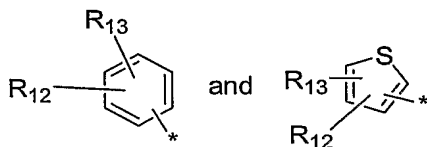
hydrogen or C<sub>1-6</sub>alkyl; and R<sub>13</sub> is chosen from halo, C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkoxy, halo-substituted-C<sub>1-6</sub>alkyl and halo-substituted-C<sub>1-6</sub>alkoxy.

5            5.            The compound of claim 1 in which A is  $-(CH_2)_2C(O)OH$ ; and R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are hydrogen.

6.            The compound of claim 5 in which n is 1 or 2; Y is selected from phenyl and benzooxazolyl; and X is a bond or selected from [1,2,4]oxadiazole, -CH<sub>2</sub>O-, -OCH<sub>2</sub>-, isoxazoles and [1,3,4]oxadiazole.

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7.            The compound of claim 6 in which R<sub>1</sub> is selected from:



15            wherein R<sub>12</sub> is selected from hydrogen, phenyl and cyclohexyl; wherein any phenyl or cyclohexyl of R<sub>12</sub> is optionally substituted with methyl; and R<sub>13</sub> is selected from trifluoromethyl, methyl and ethyl.

8.            The compound of claim 7 selected from 3-{6-[3-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-[6-(2-trifluoromethyl-biphenyl-4-yloxymethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-{5-[5-(2-trifluoromethyl-biphenyl-4-yl)-isoxazol-3-yl]-1,3-dihydro-isoindol-2-yl}-propionic acid, 3-{5-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-1,3-dihydro-isoindol-2-yl}-propionic acid, 3-{5-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,3,4]oxadiazol-2-yl]-1,3-dihydro-isoindol-2-yl}-propionic acid, 3-[2-(2-trifluoromethyl-biphenyl-4-yl)-5,7-dihydro-oxazolo[4,5-f]isoindol-6-yl]-propionic acid, 3-{7-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-{6-[5-(2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-{6-[5-(2-trifluoromethyl-biphenyl-4-yl)-

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[1,3,4]oxadiazol-2-yl]-3,4-dihydro-1H-isoquinolin-2-yl}-propionic acid, 3-[6-(3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(4-cyclohexyl-3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(2-trifluoromethyl-biphenyl-4-ylmethoxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[7-(4-cyclohexyl-3-trifluoromethyl-benzyloxy)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(4-cyclohexyl-3-methyl-phenoxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(4-cyclohexyl-3-ethyl-phenoxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid, 3-[6-(2-ethyl-biphenyl-4-yloxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid and 3-[6-(2-ethyl-3'-methyl-biphenyl-4-yloxyethyl)-3,4-dihydro-1H-isoquinolin-2-yl]-propionic acid.

9. A pharmaceutical composition comprising a therapeutically effective amount of a compound of Claim 1 in combination with a pharmaceutically acceptable excipient.

10. A method for treating a disease in an animal in which alteration of EDG/S1P receptor mediated signal transduction can prevent, inhibit or ameliorate the pathology and/or symptomology of the disease, which method comprises administering to the animal a therapeutically effective amount of a compound of Claim 1.

11. A method for preventing or treating disorders or diseases mediated by lymphocytes, for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, for inhibiting or controlling deregulated angiogenesis, or for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject comprising administering to the subject in need thereof an effective amount of a compound of claims1, or a pharmaceutically acceptable salt thereof.

12. The use of a compound of claim 1 in the manufacture of a medicament for treating a disease in an animal in which alteration of EDG/S1P receptor mediated signal transduction contributes to the pathology and/or symptomology of the disease.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/15699

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 413/10; A61K 31/4725  
US CL : 546/167; 514/307

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 546/167; 514/307

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SMITH, C.D. et al. Electrospray mass spectrometry of stable iminyl nitroxide and nitronyl nitroxide free radicals. Journal of Mass Spectrometry. 2002, Vol. 37 No. 9, pages 897-902.	8

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

01 October 2004 (01.10.2004)

Date of mailing of the international search report

20 OCT 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Janet L. Coppins

Telephone No. 571-272-1600

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/15699

### Continuation of Box II Reason 2:

In these claims, the numerous variables (e.g. R1-R13, A, X, X1, X2n, etc), their voluminous complex meanings, their seemingly endless permutations and combinations make it virtually impossible to determine the full scope and complete meaning of the claimed subject matter. As presented, the claimed subject matter cannot be regarded as being a clear and concise description for which protection is sought and as such the listed claims do not comply with the requirements of PCT article 6. Thus it is impossible to carry out a meaningful search on same. A search will be made on the first discernable invention in the claims, which is the first named compound of claim 8.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/15699

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-7 and 9-12  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Please See Continuation Sheet
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.